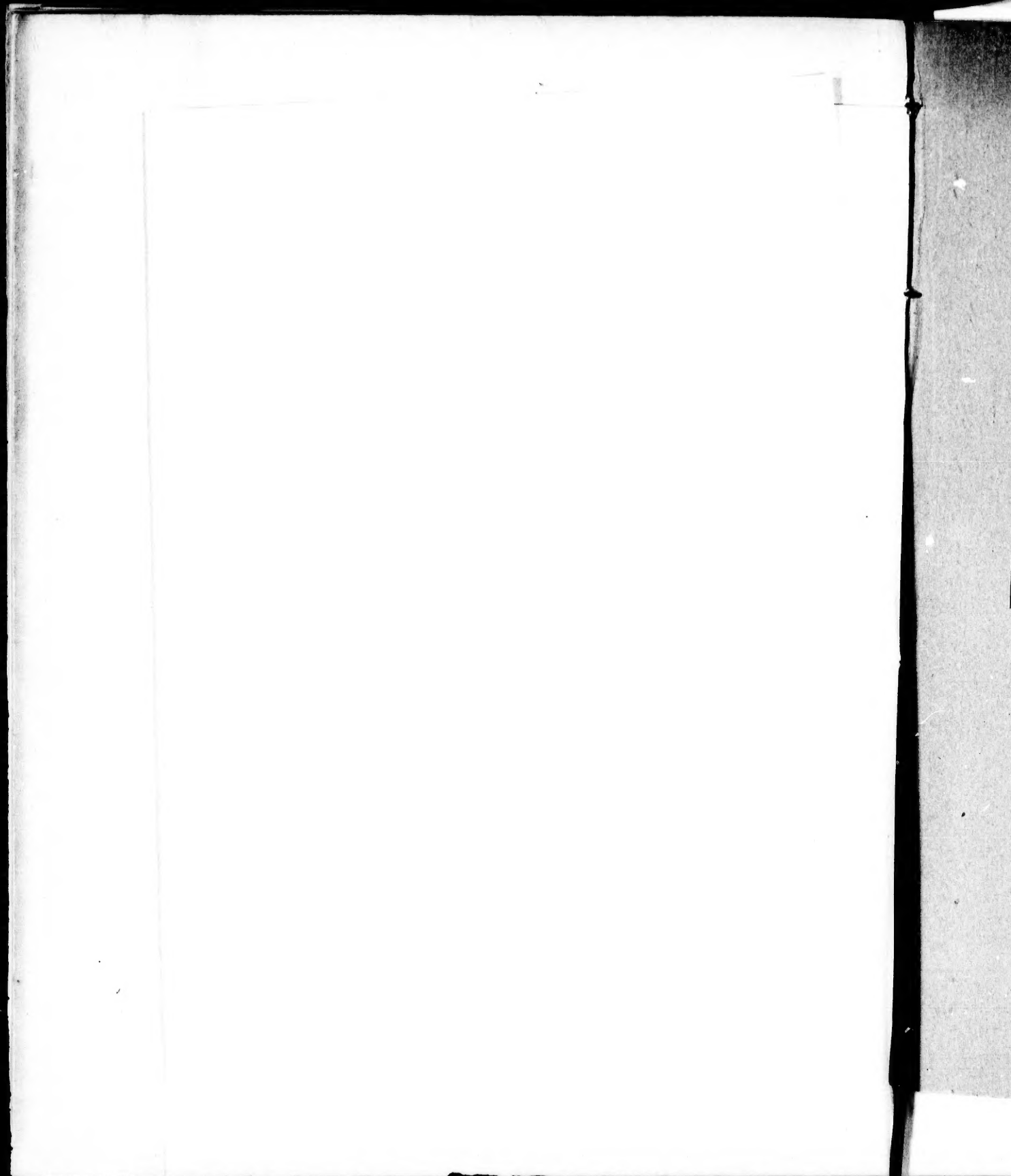




**An Experiment with the Serum
Reaction as a Test for Typhoid
Infection in Water, etc.**

BY

WYATT JOHNSTON, M. D.,

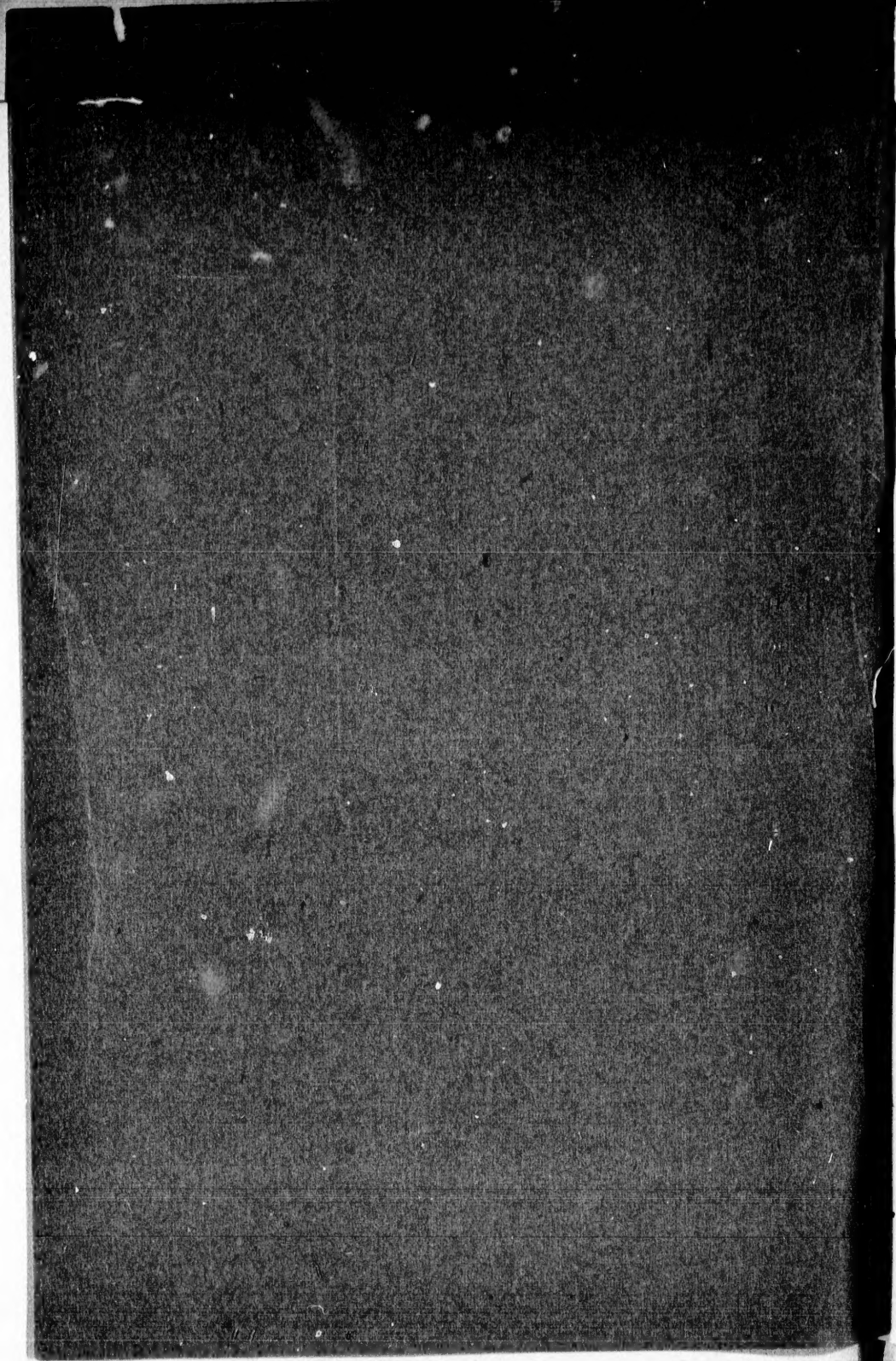
MONTREAL,

Bacteriologist to the Board of Health for the Province of
Quebec; Pathologist to the Montreal General Hospital;
Assistant Professor of Hygiene,
McGill University.

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for June 5, 1897.



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AN EXPERIMENT WITH
THE SERUM REACTION AS A TEST FOR
TYPHOID INFECTION IN WATER, ETC.*

By WYATT JOHNSTON, M. D.,

MONTREAL,
BACTERIOLOGIST TO THE BOARD OF HEALTH FOR THE PROVINCE OF QUEBEC;
PATHOLOGIST TO THE MONTREAL GENERAL HOSPITAL;
ASSISTANT PROFESSOR OF HYGIENE, MCGILL UNIVERSITY.

To say that the injection of a suitable quantity of living or dead typhoid bacilli into a suitable animal will produce a typhoid reaction in the blood of the animal is to state a well-known fact. So far as I am aware, however, no one has attempted to utilize this as a means of demonstrating typhoid infection of drinking water, milk, etc.

Wishing to test this point practically, I introduced one cubic centimetre of a typhoid bouillon culture into a flask containing two litres of tap-water from which eleven thousand colonies to the cubic centimetre grew on gelatin at room temperature. After shaking the flask, one cubic centimetre of the water thus infected was introduced into a second flask containing two litres of the same water. From this second flask, which thus represented a dilution

* Read before the Montreal Medico-surgical Society, May 4 1897.

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of one to four millions of the original bouillon culture, one cubic centimetre was placed in five cubic centimetres of ordinary peptone bouillon and grown at 37° C. for twenty-four hours. The bouillon was then sterilized for one hour at 65° C., and injected into the peritoneal cavity of a rabbit.

The animal's health remained good, except for a slight loss in weight. Its blood, examined after an interval of eight days, gave a perfectly typical reaction when tested with a typhoid culture. The blood had been tested before inoculation with negative results. The blood of a control animal inoculated with five cubic centimetres of a bouillon culture made from the same water without adding typhoid gave no reaction, nor did that of another control animal kept with the others and not inoculated.

It had occurred to me some months previously that by testing in this manner samples of suspected water and milk, typhoid infection might be demonstrated more readily than by making cultures. I tried it in the case of two samples of suspected milk in December, 1896, with negative results, but in both of these the circumstances of the case made typhoid infection seem improbable, and I thought it better to apply the test under more definite conditions.

It will be remembered that Vaughan * inoculated white rats with mixed cultures from water sediments for the purpose of demonstrating in a general way whether infective or toxic substances were present. Now that we have a definite means of recognizing the effects of the typhoid bacillus this method of investigation offers more prospect of being of permanent utility.

* *Transactions of the Society of American Physicians*, 1892.

I am now, with the aid of Dr. D. D. McTaggart, making studies as to the conditions under which a positive result may be looked for. The above experiment is cited only as an illustration of the method, possibly an exceptional one. Whether it will prove of practical use in laboratory work I am not at present able to say.

Concentration of the suspected substance by collecting the bacteria in a porcelain filter naturally renders the test more delicate, as does also the employment of specialized media for the cultures. I have found that rabbits show the reaction at an earlier stage than guinea-pigs, in some cases in two or three days after inoculation. They also have the advantage of being less susceptible than guinea-pigs to septic influences. The preliminary sterilization of the culture is not essential. It lessens to some extent the chances of obtaining a reaction from typhoid infection, but, on the other hand, it permits a larger dose to be given. By averting the danger of concurrent septic infection by other bacteria it increases the animal's chance of surviving long enough to give the reaction time to develop. Small repeated doses we know to be safer than large initial ones. With proper care a typhoid reaction can be induced without the animal's health being seriously impaired.

Capacity to produce a blood condition which will react with a genuine typhoid culture is stronger proof of a suspected organism being the genuine typhoid bacillus than capacity of a doubtful culture to react with typhoid blood, as clumping has been shown to occur with other organisms. Hence the production of the blood reaction experimentally with an organism isolated from a suspected water should not be omitted when it is necessary to operate under very rigid conditions of experiment.

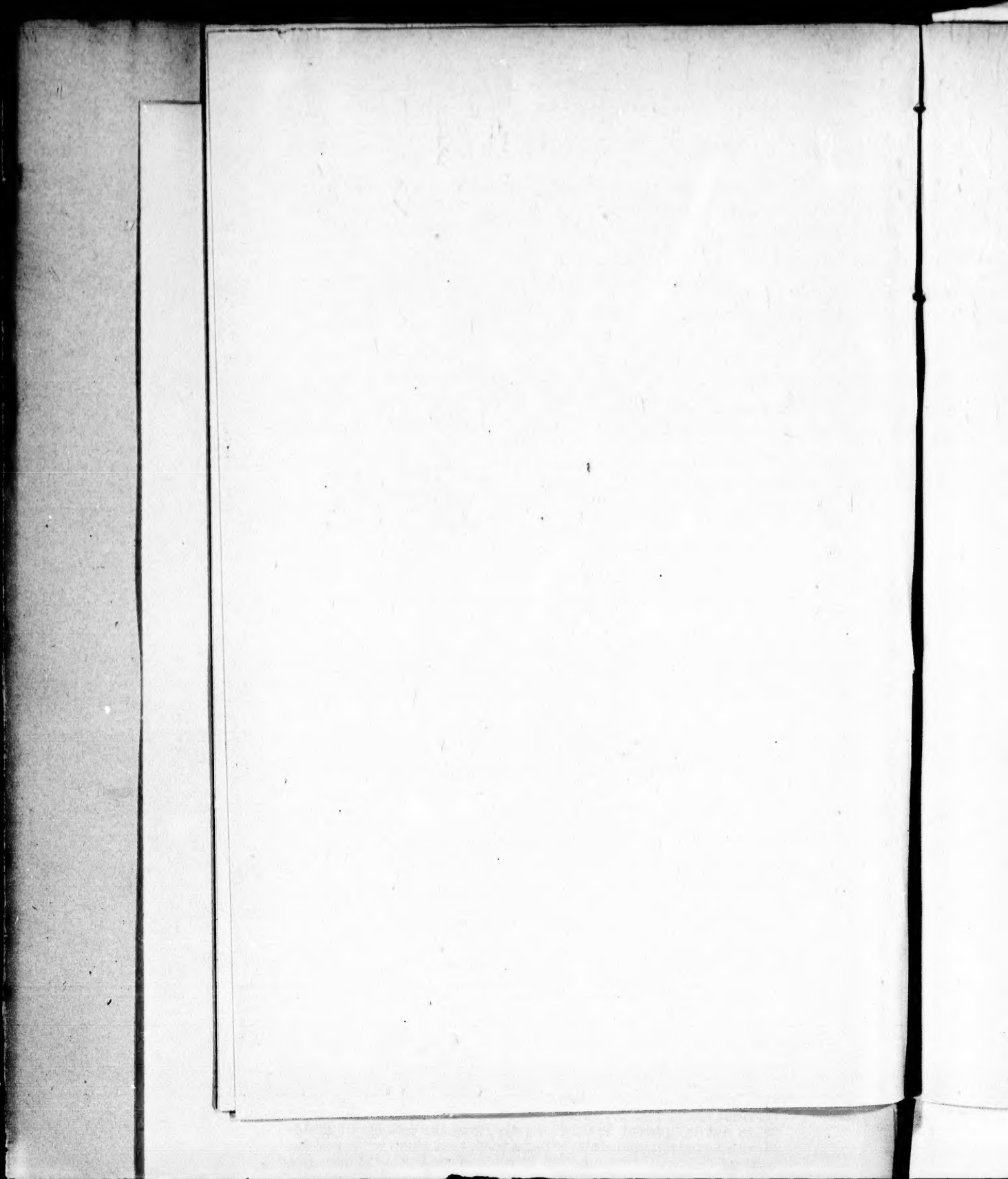
4 A TEST FOR TYPHOID INFECTION IN WATER.

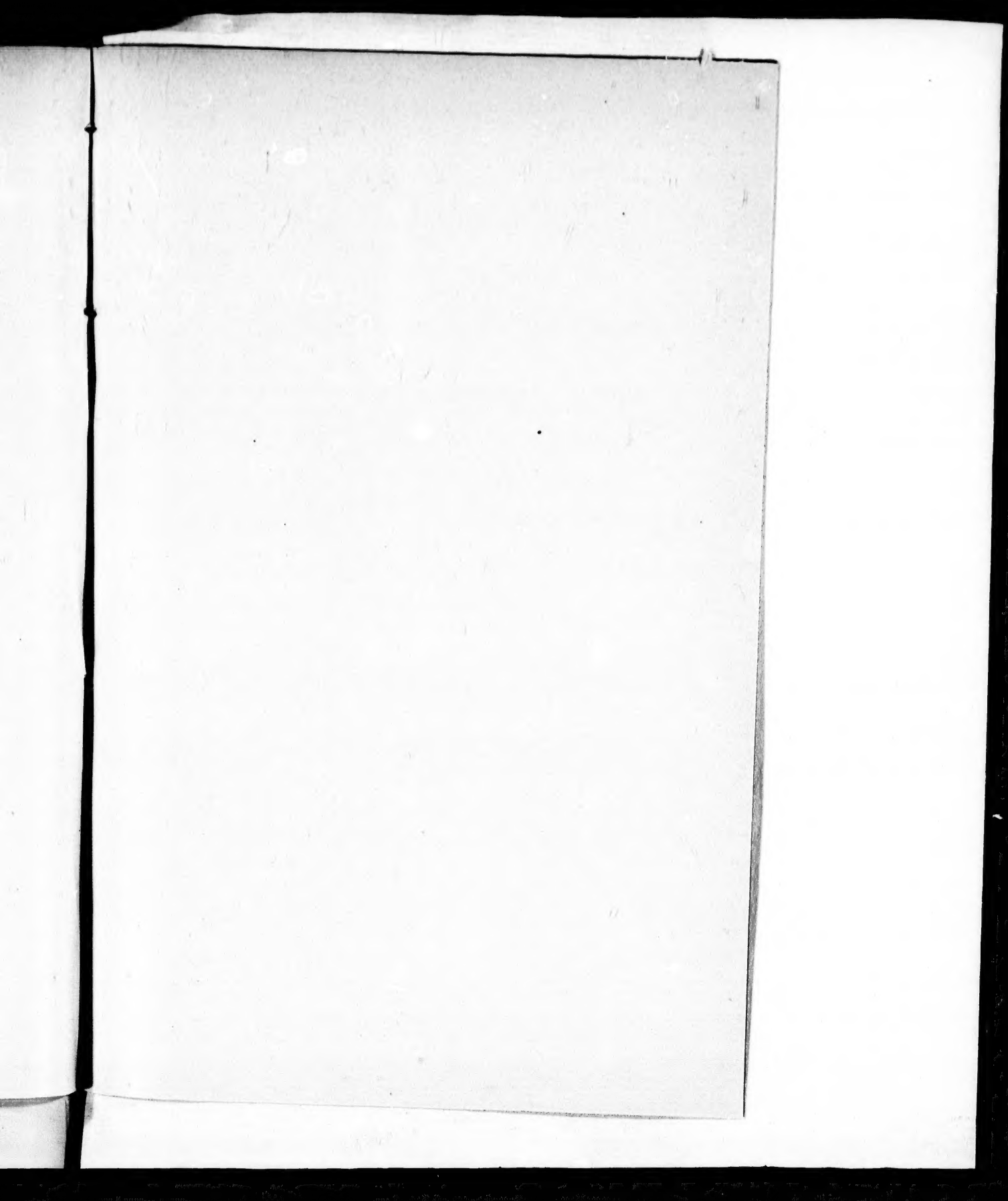
The only use to which typhoid serum reaction appears to have been applied so far by others in connection with suspected water is in the testing of organisms isolated by the usual means to see whether they react. I have already published elsewhere* short accounts of some experiments where impure twenty-four hours' bouillon cultures containing typhoid and colon bacilli were treated by adding sufficient typhoid serum to produce clumping, and then in one to two hours, when this was complete, were filtered through an inch of sand, as done in the Sedgwick-Rafter method for the quantitative microscopical analysis of water sediments. It was found that the filtrate yielded almost exclusively red colonies when grown on lactose litmus agar, whereas those obtained from the sediment were nearly all blue ones, showing that the separation of typhoid and coli by this means is rapid and complete. Care must be taken to decant or filter the culture before adding the coagulant (typhoid serum), as there is always some sediment with *Bacillus coli* at the end of the twenty-four hours' incubation. Introducing a thread or cotton filament, on which typhoid blood or serum has been dried, into the culture leads to localized clumping of the typhoid bacilli about and upon it. The paralytic effect of the typhoid serum, however, prevents this method of separation from being entirely satisfactory. I have found that for the mechanical separation to take place the typhoid bacillus must be present in considerable amount, and I have not yet worked out a satisfactory routine method of applying it to the examination of fæces or water.

* *Centralblatt für Bakteriologie*, xxi, and *British Medical Journal*, December 5, 1896 (abstract in *American Medico-surgical Bulletin*, January 10, 1897.

In the phenolized and acid bouillons recommended for typhoid isolation the typhoid clumping, as has been correctly stated by Alpers and Murray,* does not take place, but by neutralizing with soda solution I have been able to obtain it after slight delay. Alpers and Murray are not quite correct in stating that the typhoid serum reaction has only been applied to blood examinations. Elsner, Gruber, and in this country W. L. Russell have used the method in a similar manner to that mentioned by Alpers and Murray for the purpose of identifying suspected organisms isolated by culture from water or fæces. In fact, this was the chief use to which the typhoid serum reaction was applied prior to the announcement of Widal's discovery.

* *American Medico surgical Bulletin*, March 25, 1897.





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The New York Medical Journal.

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